

Association of Hypoadiponectinemia With Impaired Vasoreactivity

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Abstract—Endothelial dysfunction is a crucial feature in the evolution of atherosclerosis. Adiponectin is an adipocyte-specific plasma protein with antiatherogenic and antidiabetic properties. In the present study, we investigated the relation between adiponectin and endothelium-dependent vasodilation. We analyzed endothelial function in 202 hypertensive patients, including those who were not taking any medication. Forearm blood flow was measured by strain-gauge plethysmography. Plasma adiponectin level was highly correlated with the vasodilator response to reactive hyperemia in the total ($r=0.257$, $P<0.001$) and no-medication ($r=0.296$, $P=0.026$) groups but not with nitroglycerin-induced hyperemia, indicating that adiponectin affected endothelium-dependent vasodilation. Multiple regression analysis of data from all hypertensive patients revealed that plasma adiponectin level was independently correlated with the vasodilator response to reactive hyperemia. Vascular reactivity was also analyzed in aortic rings from adiponectin-knockout (KO) and wild-type (WT) mice. Adiponectin-KO mice showed obesity, hyperglycemia, and hypertension compared with WT mice after 4 weeks on an atherogenic diet. Endothelium-dependent vasodilation in response to acetylcholine was significantly reduced in adiponectin-KO mice compared with WT mice, although no significant difference was observed in endothelium-independent vasodilation in response to sodium nitroprusside. Our observations suggest that hypoadiponectinemia is associated with impaired endothelium-dependent vasorelaxation and that the measurement of plasma adiponectin level might be helpful as a marker of endothelial dysfunction. (*Hypertension*. 2003; 42:231-234.)

Key Words: endothelium ■ hypertension, essential ■ acetylcholine ■ adipose tissue ■ atherosclerosis

Endothelial dysfunction contributes to the development of myocardial ischemia and is a key feature in the evolution of atherosclerosis and thrombosis, the major causes of morbidity and mortality in industrialized countries.^{1,2} Endothelial dysfunction is closely associated with various pathologic conditions, including obesity, insulin resistance, diabetes mellitus, hypertension, and dyslipidemia.^{2,3} However, the underlying mechanisms have not been fully elucidated.

Adipose tissue is an important secretory organ that produces various bioactive substances known as adipocytokines, including leptin, tumor necrosis factor- α , and adiponectin, which contribute to obesity-linked metabolic and vascular diseases.^{4,5} Adiponectin is an adipocyte-specific adipocytokine, which we identified in a human adipose tissue cDNA library.⁵ We have reported that adiponectin modulated proinflammatory reactions in the vascular wall and that adiponectin-deficient mice exhibited diet-induced insulin resistance and an excessive vascular remodeling response to

injury.⁶⁻¹⁰ Clinically, hypoadiponectinemia has been identified in patients with obesity, type 2 diabetes, and coronary artery disease.^{6,11,12} These findings suggest that adiponectin has antidiabetic and antiatherogenic properties and serves as an important modulator for metabolic and vascular diseases. In the present study, we investigated the relation between adiponectin and endothelium-dependent vasodilation in humans and mice.

Methods

Study Population

Two hundred two patients with mild essential hypertension who received a medical checkup at Osaka University Hospital were enrolled in this study (Table 1). We excluded patients with severe hypertensive complications (stage III of World Health Organization), diabetes mellitus, dyslipidemia, arrhythmias, cerebrovascular disease, renal dysfunction, or malignant neoplasia, as assessed by routine physical and laboratory examinations. Brachial blood pressure was measured with a mercury sphygmomanometer after 30

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TABLE 1. Clinical Characteristics of Hypertensive Patients

Characteristic	Total (n=202)	No Medication (n=58)
Age, y	62.6±0.9	60.4±1.7
Body mass index, kg/m ²	23.7±3.3	23.6±0.5
SBP, mm Hg	142.3±1.3	153.6±2.5
DBP, mm Hg	83.0±0.8	89.2±1.6
Fasting glucose, mmol/L	5.56±0.06	5.29±0.11
Total cholesterol, mmol/L	5.00±0.05	5.19±0.12
Triglycerides, mmol/L	1.52±0.06	1.53±0.13
HDL-cholesterol, mmol/L	1.44±0.04	1.46±0.06
Log adiponectin, µg/mL	0.87±0.02	0.88±0.03

Data represent mean±SE.

minutes of rest in the supine position. Hypertension was defined as systolic blood pressure (SBP) ≥140 mm Hg, diastolic blood pressure (DBP) ≥90 mm Hg, or having received treatment for hypertension. In the present study, 58 patients were not being treated with any antihypertensive drug. Venous blood was drawn after an overnight fast. Plasma adiponectin concentration was determined by ELISA (adiponectin ELISA kit, Otsuka Pharmaceutical Co, Ltd).¹¹ Total cholesterol, triglycerides, HDL cholesterol, and glucose concentrations were determined by enzymatic methods. Body mass index was calculated as weight divided by the square of height. All subjects enrolled in this study were Japanese and gave written, informed consent. The Ethics Committee of Osaka University approved this study.

Forearm Vascular Reactivity

Forearm blood flow was analyzed by strain-gauge plethysmography (EC5R, D.E. Hokanson, Inc) as previously described.^{13,14} In brief, after cuff inflation to 300 mm Hg for 5 minutes, maximum forearm blood flow was measured as the postischemic vasodilator response to reactive hyperemia. We calculated the reactive hyperemia ratio as reactive hyperemia divided by the baseline value of forearm blood flow. After administration of 0.3 mg nitroglycerin, maximum forearm blood flow was measured as nitroglycerin-induced hyperemia. We calculated this nitroglycerin-induced hyperemia ratio as nitroglycerin-induced hyperemia divided by the baseline value of forearm blood flow. The intraobserver coefficient of variation was 2.4±1.4%, and the interobserver coefficient of variation was 2.6±1.5%.

Animals and Animal Treatment

Adiponectin-knockout (KO) mice were generated in our laboratory and backcrossed to wild-type (WT) C57BL/6J.⁹ KO and WT male mice at 8 weeks of age were fed a high-fat/high-sucrose/high-salt diet (30% fat, 15% sucrose, 8% NaCl; Oriental Yeast) for 4 weeks. SBP was measured with an automatic sphygmomanometer (MK-2000, Muromachi) at the tail artery while the animals were restrained. Blood samples were collected after an overnight fast. The experimental protocol was approved by the Ethics Review Committee for Animal Experimentation of Osaka University School of Medicine.

Vascular Relaxation Studies in Mice

The thoracic aortas of WT (n=5) or adiponectin-KO (n=5) mice were removed and placed into a modified Krebs-Henseleit buffer. Isometric tension was measured with isometric transducers (TB-611T, Nihon-Kohden). After the aortic rings (3 mm long) were equilibrated, maximal contraction was determined with 10⁻⁶ mol/L norepinephrine (Sigma Chemical Co). Relaxation was measured in response to cumulative additions of acetylcholine (ACh, Daiichi Pharmaceutical Co, Ltd; 10⁻⁹ to 10⁻⁵ mol/L) or sodium nitroprusside (SNP, Wako; 10⁻⁹ to 10⁻⁵ mol/L). Relaxation rates were expressed

as percentages of maximum relaxation (100%) induced by 10⁻⁴ mol/L papaverine (Sigma).

Statistical Analysis

Data are presented as mean±SE. Differences were analyzed by the Student unpaired *t* test. Single and multiple regression analyses were performed to analyze the correlation of the indicated parameters to the forearm vasodilator response to reactive hyperemia.

Results

Plasma Adiponectin Levels Are Positively Correlated With Endothelium-Dependent Vasodilation

We first examined the relation between plasma adiponectin concentration and forearm vascular reactivity by strain-gauge plethysmography in a total of 202 hypertensive patients, including 58 who were not taking any medication. Plasma adiponectin level was significantly correlated with the forearm vasodilator response to reactive hyperemia not only in the entire group ($r=0.257$, $P<0.001$) but also in the no-medication group ($r=0.296$, $P=0.026$; Figure 1). There was no significant correlation between plasma adiponectin level and nitroglycerin-induced hyperemia in either the entire or the no-medication group (Figure 1). The vasodilator response to reactive hyperemia was significantly correlated with plasma glucose and adiponectin levels in the entire group but with adiponectin level only in the no-medication group (Table 2). Multiple logistic regression analysis with plasma adiponectin, age, body mass index, SBP, DBP, plasma glucose, total cholesterol, triglycerides, HDL cholesterol, and medication status revealed that the vasodilator response to reactive hyperemia was independently correlated with adiponectin and glucose in the entire group and tended to associate with adiponectin in the no-medication group (Table

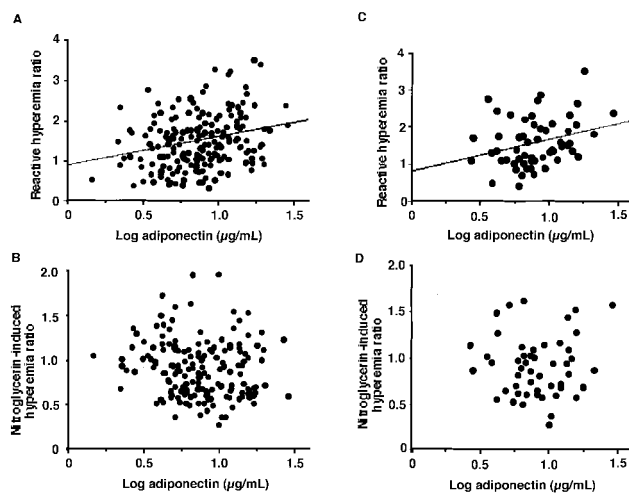


Figure 1. Positive correlation between plasma adiponectin levels and endothelium-dependent vasodilation in hypertensive patients. Relation of plasma adiponectin level with vasodilator response to reactive hyperemia in the entire (A) and no-medication (C) groups and nitroglycerin-induced hyperemia in the entire (B) and no-medication (D) groups. Reactive hyperemia ratio was expressed as reactive hyperemia divided by the baseline value of forearm blood flow. Nitroglycerin-induced hyperemia ratio was expressed as nitroglycerin-induced hyperemia divided by the baseline value of forearm blood flow.

TABLE 2. Correlation With Reactive Hyperemia by Single and Multiple Regression Analyses

	Total Group				No-Medication Group			
	Single		Multiple		Single		Multiple	
			$R^2=0.288$	$P=0.0006$			$R^2=0.561$	$P=0.1630$
	CC	P	F	P	CC	P	F	P
Age	0.124	0.080	0.607	0.438	0.188	0.158	0.447	0.515
Body mass index	0.023	0.768	3.052	0.084	0.031	0.840	0.001	0.985
SBP	0.097	0.179	2.768	0.100	0.133	0.323	1.169	0.298
DBP	0.069	0.332	0.942	0.335	0.071	0.599	0.721	0.410
Fasting glucose	0.251	0.008	4.283	0.041	0.157	0.400	0.110	0.745
Total cholesterol	0.082	0.264	0.509	0.477	0.216	0.141	0.991	0.336
Triglycerides	0.032	0.668	1.197	0.277	0.186	0.211	0.955	0.345
HDL cholesterol	0.137	0.074	0.635	0.428	0.240	0.104	0.004	0.953
Log adiponectin	0.257	<0.001	16.200	<0.001	0.296	0.026	3.191	0.095
Medication		0.258	1.873	0.175				

Data represent mean ± SE. Single and multiple regression analyses were performed to analyze the correlation of the indicated parameters to the vasodilator response to reactive hyperemia. CC indicates correlation coefficient.

2). These data indicate that plasma adiponectin level was associated with endothelium-dependent vasodilation.

Impaired Endothelium-Dependent Vasorelaxation in Adiponectin-KO Mice

We next investigated the vascular reactivity of aortic rings from adiponectin-KO and WT mice. When mice were fed the high-fat/high-sucrose/high-salt diet for 4 weeks, body weight and SBP were significantly higher in adiponectin-KO mice than in WT mice (Table 3). No significant difference was observed in pulse rate between adiponectin-KO and WT mice (Table 3). Plasma glucose was significantly higher in adiponectin-KO mice than in WT mice, although no significant difference was observed in lipid profiles (Table 3). The ACh-induced vasorelaxation was significantly reduced in adiponectin-KO mice compared with WT mice (Figure 2). In contrast, relaxation caused by SNP, which induced endothelium-independent vasodilation, did not differ between adiponectin-KO and WT mice (Figure 2).

Discussion

In the present study, we found a positive correlation between plasma adiponectin level and vasodilator response to reactive

hyperemia in hypertensive subjects and an impairment of endothelium-dependent, vascular relaxation in adiponectin-KO mice. Endothelial dysfunction is an important feature in the early stage of atherosclerosis, which is related to pathologic conditions including hypertension, diabetes mellitus, and dyslipidemia.^{2,3} After adjustment for these factors in all hypertensive patients, plasma adiponectin level could be an independent correlative

TABLE 3. Characteristics of Adiponectin-KO and WT Mice

	KO (n=5)	WT (n=5)	P
Body weight, g	29.4 ± 0.9	25.0 ± 0.6	<0.05
SBP, mm Hg	120 ± 4	103 ± 5	<0.05
Pulse rate, beats/min	714 ± 9	723 ± 15	NS
Glucose, mmol/L	9.25 ± 0.69	7.26 ± 0.37	<0.05
Total cholesterol, mmol/L	2.94 ± 0.13	3.13 ± 0.19	NS
Triglycerides, mmol/L	1.70 ± 0.18	1.76 ± 0.23	NS
HDL cholesterol, mmol/L	2.68 ± 0.16	2.77 ± 0.16	NS

Data represent mean ± SE. NS, not significant.

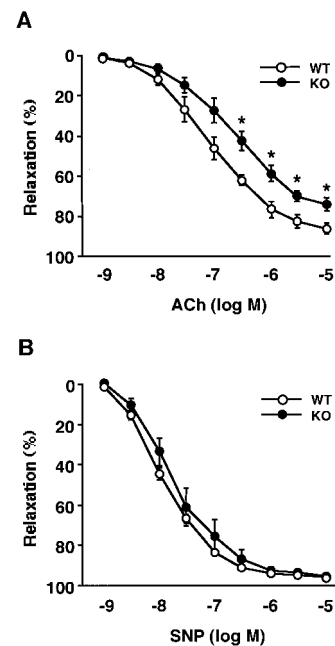


Figure 2. Impairment of endothelium-dependent vasoreactivity in adiponectin-KO mice. ACh-induced endothelium-dependent relaxations (A) and SNP-induced endothelium-independent relaxations (B) were analyzed in 10⁻⁶ mol/L norepinephrine-induced precontracted aortas of KO and WT mice. Relaxation rates were expressed as percentages of maximum relaxation (100%) induced by 10⁻⁴ mol/L papaverine. Open circles represent WT mice, and closed circles, KO mice. * P < 0.05 vs WT mice.

factor of endothelial function. We previously reported that adiponectin acts as an endogenous, biologically relevant modulator of vascular remodeling by attenuating the excessive inflammatory response in the vascular wall.^{6–10} Accordingly, plasma adiponectin level is considered a useful marker of endothelial function in hypertensive patients.

Recently, we have demonstrated that adiponectin-KO mice developed a high-fat/high-sucrose diet-induced insulin resistance as well as injury-inducible neointimal thickening.^{9,10} In this study, we investigated for the first time that adiponectin-KO mice developed obesity, hyperglycemia, hypertension, and impaired endothelium-dependent vasorelaxation compared with WT mice while being maintained on a high-fat/high-sucrose/high-salt diet. We have reported that no significant difference in metabolic parameters was observed between adiponectin-KO and WT mice while being fed a normal diet.^{9,10} In human subjects, hypoadiponectinemia is closely associated with obesity, type 2 diabetes, insulin resistance, and coronary artery disease.^{6,11,12} Therefore, only when their nutrition was overloaded did adiponectin-KO exhibit some metabolic disorders such as insulin resistance, diabetes mellitus, and hypertension, which form a common clinical background of atherogenic cardiovascular disease.

Study Limitations

The forearm vasodilator response to reactive hyperemia represents not only endothelium-dependent vasodilation but also postischemic vasoreactivity. It is still controversial whether reactive hyperemia is dependent on nitric oxide production or not. Clinically, however, measurement of the vasodilator response to reactive hyperemia is frequently used to evaluate endothelium-dependent vasorelaxation, particularly because it is a noninvasive method.^{13–15} In this study, we analyzed the reactive hyperemia ratio, which partly reflects endothelium-dependent vasodilation. An additional method might be necessary to clarify the more specific association of adiponectin with endothelium-dependent vasodilation, such as direct Ach infusion.

Perspectives

This study shows that hypoadiponectinemia not only is associated with endothelial dysfunction but also causes diet-induced hypertension. Measurement of the plasma adiponectin level therefore might be beneficial to assess the early stages of atherosclerosis. It is not possible to conclude that adiponectin directly affects endothelial function, although adiponectin is an antiatherogenic protein. Further studies can be directed toward understanding the molecular link between adiponectin and endothelial function.

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References

- Ross R. Atherosclerosis: an inflammatory disease. *N Engl J Med*. 1999;340:115–126.
- Luscher TF. The endothelium and cardiovascular disease: a complex relation. *N Engl J Med*. 1994;330:1081–1083.
- Vita JA, Keaney JF. Endothelial function: a barometer for cardiovascular risk? *Circulation*. 2002;106:640–642.
- Trayhurn P, Beattie JH. Physiological role of adipose tissue: white adipose tissue as an endocrine and secretory organ. *Proc Nutr Soc*. 2001;60:329–339.
- Maeda K, Okubo K, Shimomura I, Funahashi T, Matsuzawa Y, Matsubara K. cDNA cloning and expression of a novel adipose specific collagen-like factor, apM1 (AdiPose Most abundant Gene transcript 1). *Biochem Biophys Res Commun*. 1996;221:286–288.
- Ouchi N, Kihara S, Arita Y, Maeda K, Kuriyama H, Okamoto Y, Hotta K, Nishida M, Takahashi M, Nakamura T, Yamashita S, Funahashi T, Matsuzawa Y. Novel modulator for endothelial adhesion molecules: adipocyte-derived plasma protein adiponectin. *Circulation*. 1999;100:2473–2476.
- Ouchi N, Kihara S, Arita Y, Nishida M, Matsuyama A, Okamoto Y, Ishigami M, Kuriyama H, Kishida K, Nishizawa H, Hotta K, Muraguchi M, Ohmoto Y, Yamashita S, Funahashi T, Matsuzawa Y. Adipocyte-derived plasma protein, adiponectin, suppresses lipid accumulation and class A scavenger receptor expression in human monocyte-derived macrophages. *Circulation*. 2001;103:1057–1063.
- Okamoto Y, Kihara S, Ouchi N, Nishida M, Arita Y, Kumada M, Ohashi K, Sakai N, Shimomura I, Kobayashi H, Terasaka N, Inaba T, Funahashi T, Matsuzawa Y. Adiponectin reduces atherosclerosis in apolipoprotein E-deficient mice. *Circulation*. 2002;106:2767–2770.
- Maeda N, Shimomura I, Kishida K, Nishizawa H, Matsuda M, Nagaretani H, Furuyama N, Kondo H, Takahashi M, Arita Y, Komuro R, Ouchi N, Kihara S, Tochino Y, Okutomi K, Horie M, Takeda S, Aoyama T, Funahashi T, Matsuzawa Y. Diet-induced insulin resistance in mice lacking adiponectin/ACRP30. *Nat Med*. 2002;8:731–737.
- Matsuda M, Shimomura I, Sata M, Arita Y, Nishida M, Maeda N, Kumada M, Okamoto Y, Nagaretani H, Nishizawa H, Kishida K, Komuro R, Ouchi N, Kihara S, Nagai R, Funahashi T, Matsuzawa Y. Role of adiponectin in preventing vascular stenosis: the missing link of adipovascular axis. *J Biol Chem*. 2002;277:37487–37491.
- Arita Y, Kihara S, Ouchi N, Takahashi M, Maeda K, Miyagawa J, Hotta K, Shimomura I, Nakamura T, Miyaoka K, Kuriyama H, Nishida M, Yamashita S, Okubo K, Matsubara K, Muraguchi M, Ohmoto Y, Funahashi T, Matsuzawa Y. Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. *Biochem Biophys Res Commun*. 1999;257:79–83.
- Hotta K, Funahashi T, Arita Y, Takahashi M, Matsuda M, Okamoto Y, Iwahashi H, Kuriyama H, Ouchi N, Maeda K, Nishida M, Kihara S, Sakai N, Nakajima T, Hasegawa K, Muraguchi M, Ohmoto Y, Nakamura T, Yamashita S, Hanafusa T, Matsuzawa Y. Plasma concentrations of a novel, adipose-specific protein, adiponectin, in type 2 diabetic patients. *Arterioscler Thromb Vasc Biol*. 2000;20:1595–1599.
- Komai N, Ohishi M, Morishita R, Moriguchi A, Kaibe M, Matsumoto K, Rakugi H, Higaki J, Ogihara T. Serum hepatocyte growth factor concentration is correlated to the forearm vasodilator response in hypertensive patients. *Am J Hypertens*. 2002;15:499–506.
- Sanada M, Higashi Y, Nakagawa K, Sakaki S, Kodama I, Tsuda M, Nagai N, Ohama K. Relationship between the angiotensin-converting enzyme genotype and the forearm vasodilator response to estrogen replacement therapy in postmenopausal women. *J Am Coll Cardiol*. 2001;37:1529–1535.
- Celermajer DS, Sorensen KE, Gooch VM, Spiegelhalter DJ, Miller OI, Sullivan ID, Lloyd JK, Deanfield JE. Non-invasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis. *Lancet*. 1992;340:1111–1115.

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